

# The CREST Biorepository: A Tool for Molecular Epidemiology and Translational Studies on Malignant Mesothelioma, Lung Cancer, and Other Respiratory Tract Diseases

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## Abstract

**Objectives:** The Cancer of RESpiratory Tract (CREST) biorepository was established to investigate biological mechanisms and to develop tools and strategies for primary and secondary prevention of respiratory tract cancer. The CREST biorepository is focused on pleural malignant mesothelioma, a rare and severe cancer linked to asbestos exposure whose incidence is particularly high in the Ligurian region.

**Methods:** The CREST biorepository includes biological specimens from (a) patients with pleural malignant mesothelioma and lung cancer, (b) patients with non-neoplastic respiratory conditions, and (c) control subjects. Whole blood, plasma, serum, lymphocytes, pleural fluid, saliva, and biopsies are collected, and a questionnaire is administered. Collection, transportation, and storage are done according to international standards.

**Results:** As of January 31, 2008, the overall number of subjects recruited was 1,590 (446 lung cancer, 209

pleural malignant mesothelioma, and 935 controls). The biorepository includes a total of 10,055 aliquots (4,741 serum; 3,082 plasma; 1,599 whole blood; 633 pleural fluid; and 561 lymphocytes) and 107 biopsies. Demographic, clinical, and epidemiologic information is collected for each subject and processed in a dedicated database.

**Conclusions:** The CREST biorepository is a valuable tool for molecular epidemiology and translational studies. This structure relies on a network of contacts with local health districts that allows for an active search for patients. This is a particularly efficient approach, especially when the object of the study is a rare cancer type. The CREST experience suggests that the presence of limited resources can be overcome by the biorepository specialization, the high quality of the epidemiologic information, and the variety of samples. (Cancer Epidemiol Biomarkers Prev 2008;17(11):3013–9)

## Introduction

The inclusion of biomarkers in population-based studies is at the basis of molecular epidemiology. The measurement of biomarkers in blood or tissue specimens has become an integral component of translational cancer research, with applications to studies on cancer etiology, treatment, and prognosis, including early cancer detection (1).

However, even when a complete molecular profile of each study subject is available, its use may be of limited use if the role of host factors, disease features, carcinogen exposures and lifestyle, response to therapy, and survival are not taken into account (2). Biorepo-

sitories, with an associated archive of epidemiologic data, are among the most powerful and essential resources for molecular epidemiology and translational studies.

Although the role of lung cancer as the leading cause of death from cancer in Western countries is well known (3), interest in pleural malignant mesothelioma has recently increased because of the expected peak of cancer incidence in the second decade of the new century (4). Asbestos exposure is a well-recognized risk factor for both tumors, characterized by high death rates and the low success of conventional therapies in improving the life expectancy of patients (5). With this in mind, the use of biomarkers in clinical and etiologic studies may provide a valuable contribution.

The incidence of pleural malignant mesothelioma is particularly high in the Liguria region, because of a high concentration of asbestos-related industries, including extensive shipyard activity. From 1996 to 2002, the standardized age (European population) incidence rates in this area according to the Mesothelioma Registry of Liguria were 8.51 and 1.43 per 100,000 males and females, respectively (the highest observed rate reached

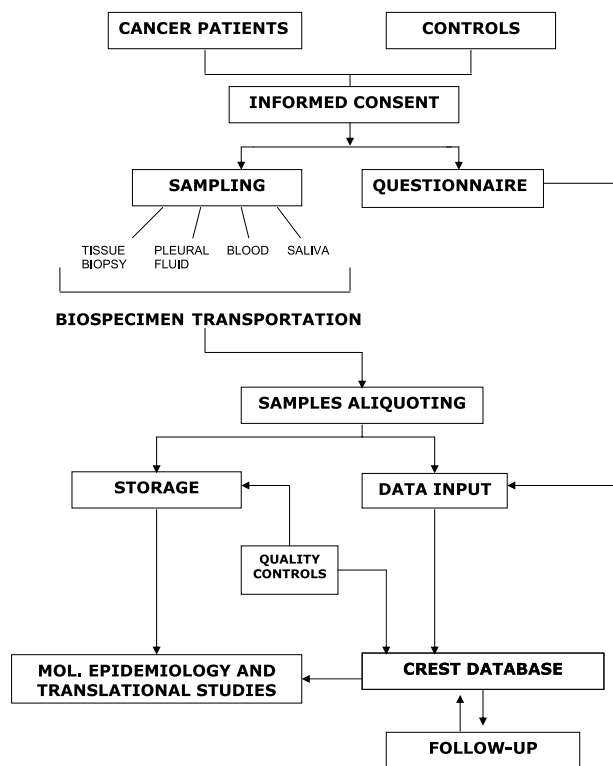
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**Figure 1.** Schematic representation of the CREST biorepository organization.

the value of 13.7 for males in La Spezia). These figures are ~10-fold higher than those reported for most industrialized countries where asbestos was used widely (6).

For locations such as the lung and the pleura, tumor specimens are difficult to obtain. The use of surrogate tissues offers a great potential to epidemiologic studies (further supported by the developing evidence associating primary DNA or protein damages observed in tumor tissues with similar alterations in surrogate tissues) and benefits from the easy collection and storage of blood or exfoliated cells. The number of association studies between DNA polymorphisms and cancer phenotype has been boosted thanks to the extended availability of biorepositories and their prompt access to patients' DNA.

The most popular study design in molecular epidemiology is, by large, the case-control approach, which is the most efficient in using samples from biorepositories. Case-control studies have proven to be especially efficient in combination with cohort studies, when biological samples are stored at the beginning of the cohort recruitment, using the so-called nested design.

In this article we describe the main methodologic features of the Cancer of the RESpiratory Tract (CREST) project. This effort aims to establish a comprehensive resource to facilitate the synergistic interplay between the information provided by biospecimens and correspondent epidemiologic and clinical data. The CREST biorepository is to be used in molecular epidemiology

and translational studies of respiratory tract cancer, with special focus on pleural malignant mesothelioma. CREST is a part of the Biological Resource Center of the National Cancer Research Institute, Genoa.

## Materials and Methods

The CREST biorepository was established in 1996 at the National Cancer Research Institute, Genoa. The initiative was planned to optimize the conduct of multicenter molecular epidemiology studies for the development and validation of biomarkers for the primary and secondary prevention of pleural malignant mesothelioma and lung cancer. The other aims of the biorepository are to support epidemiologic studies on exposure to airborne carcinogens, identify subgroups of susceptible individuals, and estimate the cancer risk associated with early molecular events.

In Fig. 1, the schematic representation of the CREST biorepository organization is shown.

**Subjects.** The CREST biorepository includes specimens donated by subjects participating in studies coordinated by, or done in scientific partnerships with, the Units of Molecular Epidemiology and Epidemiology and Biostatistics of the National Cancer Research Institute of Genoa. All subjects are resident in the Liguria Region or in other areas considered at risk of neoplastic or nonneoplastic respiratory diseases. Specimens archived in the CREST biorepository are classified according to the following categories of subjects:

*Patients with respiratory tract cancer.* Patients who have been diagnosed with lung cancer or pleural malignant mesothelioma and that are sampled before any treatment, including surgery. Samples are collected within the framework of ad hoc molecular epidemiology studies or are obtained through a collaborative regional network of pneumologic departments.

*Subjects with benign respiratory pathology.* Patients with nonneoplastic respiratory conditions (mostly chronic obstructive pulmonary disease and asbestosis), often hospitalized at the same clinical departments where cancer cases are selected.

*Controls.* Patients hospitalized for nonneoplastic and nonrespiratory conditions in orthopedic or ophthalmology departments. Other reference subjects are recruited from blood donors, from social and recreational clubs and homes for the elderly located in the same geographic area where cancer patients are hospitalized. Community outreach programs, including information provided through local TV and newspapers, are occasionally carried out to increase compliance in this group.

**Informed Consent.** Before contributing any kind of biological sample, all subjects are requested to sign an informed consent. Each donor receives detailed information about the purposes of the study, and any risk, discomfort, or potential benefit deriving from the participation in the study. The lack of immediate benefits for the participant is clearly stated, whereas the contribution of the study to improving prevention and

early diagnosis of respiratory tract pathologies is emphasized. Consent is collected by a trained physician (for subjects affected by cancer or a nonneoplastic respiratory disease) or by CREST staff (for control subjects). Special emphasis is given to the current and future use of the samples, including the possibility of unforeseen applications. All subjects are also asked to consent to an individual follow-up and are informed about the right to withdraw their consent to use their biological material at any time. The subject who agrees to enter the study signs the official consent, declaring that he/she understands the aims of the study and voluntarily agrees to participate. The subject is then encouraged to keep a copy of the signed consent as an information source throughout the course of the study.

**Ethical Issues.** Approval for the recruitment has been obtained from the Ethical Board. All ethically relevant procedures have been planned in agreement with international guidelines for biorepositories (7, 8). Individual data included in the CREST databank were treated in accordance with the Italian privacy regulation (DL 196/2003).

**Questionnaire.** After signing the informed consent, each participant is asked to complete a structured questionnaire. The questionnaire includes sociodemographic data, residence history, occupational history with dates, lifestyle information (tobacco smoking and diet), medical history, and family history of cancer in first-degree relatives. Questionnaires are then processed and valid data are stored in the CREST database.

**The CREST Database.** A customized Access database has been developed to store specimen information and to manage the link with clinical and epidemiologic data. The interactive structure of the database allows the adding of new types of specimens and information as research interests grow and diversify. It is possible to make queries across groups to facilitate any combination of sample retrieval and to summarize available data for the preparation of periodic reports. All samples stored in the biorepository are stripped of any personal information and can be identified only through the use of a unique code. Access to personal data is restricted to biorepository curators. The management of authorized requests for biological material and associated epidemiologic data is in the charge of authorized data managers who enter the database using the code.

**Clinical Features.** Clinical features concerning all hospitalized patients are collected through a linkage with institutional medical records. Histologic parameters for patients with cancer diagnosis are collected from the same source or from pathology records. To evaluate the effect of clinical parameters on progression and survival of the disease, subjects are actively followed up by searching clinical record archives. A collaboration has been established with local cancer registries, namely the Genoa Cancer Registry and the Regional Mesothelioma Registry, in the area where the CREST biorepository is located.

**Specimens.** Several different specimens are included in the repository, i.e., serum, plasma, cultured lymphocytes, whole blood, saliva, pleural fluid, and biopsies. Specimen tubes are labeled by the CREST staff with a

unique numerical code representing the identity code for each subject and ensuring blindness and respect of privacy. The same code is also reported on the questionnaire forms and is the key to link the specimen to the donor.

Specimens are collected in coded vials by medical or paramedical personnel of the clinical departments participating in the CREST project. When blood samples are not easily available, saliva is collected with a special device. The biological material is transported in a thermal bag by CREST staff from the peripheral centers to the biorepository laboratories. When necessary, a special homologated shockproof container (Biobox Dewar; Karlsruhe Glastechnisches Werk) is used, which allows for the storage of samples in a wide range of temperatures (from  $-70^{\circ}\text{C}$  to  $+4^{\circ}\text{C}$ ) and guarantees the integrity of biological features. Specimens are never transferred by mail because recent postal security practices, including irradiation, could affect their quality. The transfer protocol states that biological samples received by biorepository laboratories should be registered, processed, and stored the same day within 4 h from the collection. The laboratories where samples are aliquoted and stored are certified by the International Organization for Standardization. A specific protocol describing the procedures for collection, transportation to the biorepository, and storage has been developed in agreement with international standards for biorepositories (7, 9-12).

Five freezers are available for the storage of biospecimens collected. Whenever possible, each batch is divided into aliquots which are stored in different freezers and in different locations. A backup power system is available (motor generator) working up to 48 h in case of power failure. All freezers are connected to a networked alarm system operating 24 hours a day, 7 days a week. A person from the National Cancer Research Institute staff monitoring the whole refrigeration system has been identified, and procedures are being developed to alert the key staff of the biorepository in case of alarm.

The standard set of samples collected from each patient, whenever allowed by therapeutic protocol, patient conditions, and the amount of the specimen collected, is made up of the following:

*Serum.* Peripheral blood is collected by standard venipuncture into a red top Collect Vacutainer tube (6 ml BD Vacutainer Systems) with no additive and transported at room temperature. The sample is allowed to clot for at least 30 to 60 min, then the blood tube is spun in a standard centrifuge for 10 min at 3,500 rpm. After spinning, serum is down off from the tube in 0.5-ml aliquots and stored in a  $-80^{\circ}\text{C}$  freezer.

*Plasma.* Peripheral blood is collected by standard venipuncture into a blue top Collect Vacutainer tube (7 ml BD Vacutainer Systems) with lithium-heparin or into a purple top Collect Vacutainer tube (6 mL BD Vacutainer Systems) with EDTA additive and transported at room temperature. The blood tube is spun in a standard centrifuge for 10 min at 3,500 rpm. After spinning, plasma is down off from the tube in 0.5-ml aliquots and stored in a  $-80^{\circ}\text{C}$  freezer.

*Lymphocytes.* Half of the blood collected into the blue top Collect Vacutainer tube with lithium-heparine

additive is used for viable lymphocytes extraction. Cultured lymphocytes are assayed with the modified cytokinesis-blocked method of Fenech and Morley to measure micronuclei frequency (13).

*Whole blood.* Peripheral blood is collected by standard venipuncture technique into an azure top Collect Vacutainer (2.7 ml BD Diagnostics) citrate-stabilized blood, transported at room temperature. Aliquots of 0.5 mL of whole blood are stored in a  $-80^{\circ}\text{C}$  freezer. DNA may be extracted later on demand with the Sambrook method modified (14).

*Saliva.* When blood samples of control subjects are not available, saliva is collected as an alternative DNA source using a special kit (Oragene DNA Self-Collection Kit). DNA in saliva comes from epithelial cells and WBC found in the mouth. Samples are transported and stored at room temperature and subsequently DNA is extracted with the Oragene DNA Purification Protocol. Validation experiments have shown that DNA from saliva and from blood are equivalent (15, 16).

*Pleural fluid.* Pleural fluid is collected in 50-ml Falcon tubes, transported at room temperature, and centrifuged at 1,000 rpm for 10 min to pellet the cells. A standard number of 10 aliquots of supernatant are then kept frozen ( $-80^{\circ}\text{C}$ ) until use.

*Biopsies.* Cancer tissue is collected by a surgeon during thoracoscopy or surgery according to standard clinical practice. Samples are collected in microvials frozen in containers with dry ice and ethanol (Biobox Dewar; Karlsruher Glastechnisches Werk) and locally stored at  $-80^{\circ}\text{C}$ . Collection, transportation from collaborating hospitals to the biorepository, and storage take place within 4 h.

**Quality Control.** The laboratories where samples are aliquoted and stored are certified by the International Organization for Standardization. A standard operating procedure manual has been developed and is regularly updated. Major procedures for quality assurance and data management, collection and transportation of the specimens, specimen processing, control of instrument or equipment performance, quality control of the stored samples, specimen distribution, and staff training and internal audits, are included.

A quality control process has been planned to check the accuracy and completeness of epidemiologic data stored into the CREST database. Every 6 mo a trained computer assistant runs an automated check of the Access database. Other quality control procedures have been designed to confirm, through a double check, for any new subject included in the bank, that the sample and the information from the questionnaire are properly linked by the unique code. A back-up of all available information is done whenever a new individual or new information is included in the bank.

Specimen quality, storage vial integrity, aliquot location, and related electronic records are checked annually. Specimen quality is verified yearly by confirming bioavailability and measuring biodegradation in at least 1% of the stored samples.

**Use of Specimens and Data.** Applications for the scientific use of stored specimens and epidemiologic/

clinical data are reviewed and authorized by the biorepository steering committee. The committee is composed of the biorepository coordinator, two researchers in staff to the CREST project, and two invited collaborators (one is a medical doctor) among those contributing samples to the bank. Applications can be presented by any research group interested in respiratory cancers, and are evaluated according to the scientific interest of the proposal, the qualification of the proponents, the presence of ethical concerns, and the availability of aliquots for the samples requested. The inclusion in the study group of researchers from the biorepository is requested to recognize the effort in selecting samples and providing high standards in the quality of biological samples and epidemiologic/clinical data.

Specimens and data stored in the CREST biorepository have been used mostly for ad hoc molecular epidemiology and translational studies. Participation in large international disease consortia is currently ongoing. In particular, specimen and data contribution to the International Lung Cancer Consortium, an international group of lung cancer researchers established with the aim of sharing comparable data from ongoing lung cancer case-control and cohort studies, is in progress.<sup>9</sup> Additionally, DNA from pleural malignant mesothelioma patients and controls will be used to contribute to a large international genome-wide association study on this neoplasm.

**Resources.** To ensure a service of sample storing without interruptions and to maintain the qualitative standards set in the protocols, adequate availability of resources and personnel is required. Unfortunately, the availability of funds to support research is limited, and existing resources in public institutions can be used and relocated to this initiative. An efficient approach for these low-investment initiatives is the delimitation to specific cancer types. This choice makes it possible to optimize the phase of sample collection, which is directed to a specific clinical setting and reduces the number of specimens necessary to reach a statistically meaningful size. A useful biorepository can be created anyway, maintaining high-quality levels despite the limited investment. Funds for subject recruitment and sample collection are generally requested when applying for scientific projects grants.

The CREST biorepository has become part of a larger initiative of the National Cancer Research Institute, which has set up the Biological Resource Center, a project aimed at ensuring that biological material collection is developed within appropriate legal, ethical, clinical, and technical guidelines. The CREST biorepository staff is coordinated by a Ph.D. researcher who is in charge of formulating collection strategies, ensuring the proper consideration of legal and ethical issues, keeping contacts with collaborating centers, and managing personnel and equipment resources. Other personnel include two senior researchers who dedicate part of their time to coordinate; update the written procedures on specimen collection, transportation, storage, and scientific use; perform quality controls; and design new

<sup>9</sup> www.ilcco.net

studies on the CREST biorepository. A laboratory personnel (part-time) is in charge of processing, aliquoting, and archiving samples; a data manager (part-time) is responsible for data entry, queries, quality control on data input, follow-up, and the provision of statistics for periodic reports; and an interviewer (part-time) obtains informed consent, administers the questionnaire, and transports samples to the laboratories.

The resources necessary to maintain the biorepository are provided by the National Cancer Research Institute of Genoa and the University of Genoa, which pay for part of the personnel salaries (two scientists, one laboratory personnel, one data manager), for the freezer surveillance system (shared with other research groups), and for general costs (telephone, power, heating, freezer maintenance, etc.). All other costs and salaries are covered by external grants (the main sponsor is the Fondazione Buzzi ONLUS, Casale M., Italy). A general estimate of the yearly cost of maintaining the biorepository is about EUR 130,000 for the staff, EUR 20,000 for general expenses, and EUR 20,000 for costs directly linked to sample collection (travel, courier, etc.), bank maintenance (software update, laptops, etc.), and the scientific activity of the bank (missions, publication costs, ad hoc studies, etc.). The overall amount can be estimated at about EUR 170,000.

The future of the biorepository is linked to the National Cancer Research Institute's commitment to create a quality environment networking the institutional biorepository to the small biorepositories through the Biological Resource Center and by active participation in the Biobanking and Biomolecular Resources Research Infrastructure project within the European Union 7th framework program. The project responds to the European Strategy Forum on Research Infrastructures Roadmap for a pan-European and broadly accessible network of existing and *de novo* biorepositories and biomolecular resources. Samples from patients and healthy subjects will be included in the infrastructure along with molecular genomic resources and bioinformatics tools to optimally exploit this resource for global biomedical research.

**CREST Biorepository Performance.** The annual number of biospecimens collected varies greatly according to the compliance of collaborating centers and to the success of external grant applications. As of January 31, 2008, the overall number of recruited subjects was 1,590 (446 lung cancer, 209 pleural malignant mesothelioma, and 935 controls). The biorepository included a total of 10,055 aliquots (4,741 serum aliquots; 3,082 plasma aliquots; 1,599 aliquots for DNA extraction; 633 pleural fluid aliquots; and 561 lymphocytes aliquots), and 107 biopsies. The biorepository currently receives about 5 sample sets per week.

Several molecular epidemiology and translational studies have been already done based on the CREST biorepository. The endpoints measured in these studies included levels of circulating proteins (HER-2/neu, mesothelin, osteopontin, mutated K-ras), growth factors (PDGF-AB, HGF, EGF, SCF) or antibodies (anti-p53), erythrocyte glycoprotein-A variants, DNA adducts, micronuclei, DNA repair activities, SV40, and single-nucleotide polymorphisms in metabolic, DNA repair

or other genes. A list of the published articles is reported in the references (17-34).

Given the biorepository's aims, which are essentially focused on promoting collaborative research, groups interested in biological samples to study outcomes of the respiratory tract carcinogenesis are invited to contact us at the following address: donatella.ugolini@istge.it.

**Other Biorepositories Including Mesothelioma Samples.** In the United States, the National Mesothelioma Virtual Bank<sup>10</sup> has been created, a project of the Departments of Biomedical Informatics, Epidemiology, and Pathology at the University of Pittsburgh. This initiative, funded by grants from the National Institute of Occupational Health and Safety and the Centers for Disease Control and Prevention, has been created to provide institutions and medical researchers involved in investigating mesothelioma with a resource of mesothelioma tissue samples that are clinically annotated and collected in an accessible database. Nine hundred biospecimens, including paraffin-embedded, fresh-frozen blood and DNA samples, are available. For each case, available data include demographics, epidemiologic data, clinical history, pathology data including block level annotation, and follow-up data including treatment, recurrence, and vital status (35).

Another ongoing project is the Australian Mesothelioma Tissue Bank. This biobank is part of the Australasian Biospecimen Network-Oncology project funded by a grant from the Australian National Health and Medical Research Council.<sup>11</sup>

Another biorepository of human mesothelioma biospecimens has been developed in Italy, in the city of Casale Monferrato. Blood samples of about 200 cases of mesothelioma and 220 controls are available. All subjects have been interviewed on their lifelong occupational and residential history by a trained interviewer using a validated questionnaire (36).

**Conclusions.** This is a brief overview of the CREST biorepository project, aimed at providing a resource for molecular epidemiology and translational research on lung and pleural cancer. The biorepository's added value can be summarized in four major issues: (a) the collection, simultaneously with the biological sample, of an extensive set of individual clinical and epidemiologic data that facilitates the conduct of molecular epidemiology and translational studies; (b) the selection of a homogenous set of cancers, i.e., lung cancer and pleural malignant mesothelioma, which share common risk factors and are treated at the same departments, allowing an easier collection of specimens and administration of questionnaire; (c) the availability of epidemiologic data and biological samples from subjects with nonneoplastic respiratory diseases, improving the potential to understand the various steps of the cancer pathway and to implement primary and secondary prevention strategies; and (d) the recruitment of reference subjects conducted in two different settings, hospital and volunteers, which

<sup>10</sup> <http://www.mesotissue.org>

<sup>11</sup> <http://www.abrn.net>

makes it possible to reduce the effect of a possible selection bias.

The availability of a variety of biological samples combined with epidemiologic and clinical data in different study groups allows for a wide range of investigations covering different time frames of the natural history of the disease. Among these, the most promising development involves the role of genetic factors in the etiology of the disease and the effect of individual susceptibility. Recent achievements by genome-wide association studies have boosted the importance of biorepositories, and have contributed to the development of high-throughput, cost-effective methods for genotyping. Among the most exciting results is the identification of the first genetic polymorphism consistently associated with lung cancer (37-39). Other high-priority uses of stored samples include the identification of synergism among risk factors (e.g., asbestos and SV40), the development of new biomarkers for early diagnosis, and the choice of new targets to improve therapeutic performances. The adoption of freezing procedures compatible with the preservation of RNA, besides being a more clinically oriented research, will allow the exploration of the use of stored samples to test future hypotheses concerning disease risk factors and early biomarkers of exposure.

New methods and procedures are under evaluation to improve the biorepository's performance, including a new system of sample labeling with a hospital system-generated barcode ID number and the adoption of a mechanical system for specimen manipulation. This will require additional investments in terms of human and material resources, and it may perhaps imply the request of a financial contribution to research groups asking access to stored specimens.

The choice of focusing sample collection to respiratory tract cancers and related controls and administering an extensive questionnaire, has raised a number of critical issues. A high degree of flexibility is requested from the CREST staff directly involved in sample collection, depending on diagnostic procedures and the stage of the disease. In particular, sample collection during delicate clinical procedures such as thoracoscopy or bronchoscopy may encounter difficulty in timing and unpredictable results. On the other hand, the questionnaire is sometimes difficult to administer to patients in bad condition. A further challenge has been posed by the old age of cancer cases, especially those affected by pleural malignant mesothelioma, which entails a difficult search for controls of similar age.

In conclusion, the experience of the CREST project confirms the importance of biorepositories for the successful conduct of molecular epidemiology and translational studies on respiratory tract diseases. A structure capable of actively searching for patients, based on an affordable network of contacts in a high incidence area, is extremely efficient. This approach is particularly suitable when the study object is a rare cancer as pleural malignant mesothelioma, and the CREST biorepository has one of the largest collections of pleural malignant mesothelioma specimens described in literature.

The problem of limited resources can be overcome by the biorepository through specialization, focusing on a

restricted number of tumor sites that are homogeneous for risk factors and may be collected in few clinical centers. Finally, the relatively low number of specimens available in a small biorepository should be compensated by the high quality of the linked clinical and epidemiologic information and by the diversity of sampled tissues.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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